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BIOLUMINESCENT BACTERIA OF THE BLACK SEA AND SEA OF AZOV

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The aim of the present study was to isolate bioluminescent strains from the northern Black Sea and Sea of Azov, analyze their morphological and biochemical characteristics, and identify them based on 16S rRNA, *recA*, and *gyrB* gene sequences. Nine isolates were isolated from hydrobionts, and twelve, from seawater. Results of biochemical and molecular genetic identification revealed that isolated luminous strains represent the genera *Vibrio*, *Aliivibrio*, and *Photobacterium*. All five cultivated luminescent strains isolated from water and hydrobionts of the Sea of Azov belong to the species *Photobacterium leiognathi*. Cultivated luminous bacteria of the Black Sea are assigned to the genera *Aliivibrio* and *Vibrio*. The genus *Aliivibrio* is represented by two *Aliivibrio fischeri* strains related to various hydrobionts. Fourteen strains of the genus *Vibrio* belong to the species *Vibrio campbellii*, *V. jasicida*, *V. harveyi*, *V. owensii*, and *V. aquamarinus* sp. nov. Thus, it was shown that taxonomic composition of the cultivated luminescent bacteria differs greatly in the Black Sea and Sea of Azov.

Keywords: luminous bacteria, identification, taxonomic composition, biodiversity, Black Sea, Sea of Azov

Currently, bacteria are among the most common model biological objects in basic and applied research. Out of them, bioluminescent bacteria are a special natural phenomenon. Those are intensively studied all over the world and used in solving various problems of biology, genetics, and biotechnology.

Bioluminescence of bacteria formed the basis of many analysis methods widely applied in practice. These are bioluminescent testing of humoral and cellular bactericidal blood systems *in vitro* and study of the characteristics of the infectious process on models *in vivo* [Deryabin, 2009]. In the practical application of bioluminescent bacteria, a significant role is played by the analysis of the integral toxicity of various natural environments. Natural and recombinant luminescent microorganisms have become a recognized tool for environmental monitoring [Baumstark-Khan et al., 2007; Chugunova et al., 2016; Ivask et al., 2007; Niu et al., 2008; Sazykin et al., 2015, 2016; Sönmez et al., 2016; Tsybulskii, Sazykina, 2010] and analysis of new substances and materials [Kovalenko et al., 2013; Kuryanov et al., 2011; Zheng et al., 2010].

Luminescent bacteria studies served as the basis for the discovery of quorum sensing – a phenomenon of general biological significance [Taga, Bassler, 2003]. First, this genetic mechanism was noted in marine luminous bacteria *Aliivibrio fischeri* and *Vibrio harveyi*; later, it was found in many other species of bacteria as a regulator of manifestations of numerous properties, including pathogenic ones. Thus, bacterial bioluminescence opens up exceptional methodological opportunities in a variety of applications

in biology, ecology, and medicine. Despite noticeable progress in the investigation of physiology, biochemistry, and genetics of luminous bacteria, many issues of their species composition and distribution in ecosystems remain unclear.

Bioluminescent bacteria are widespread in nature [Ast et al., 2009; Baumann, et al., 1984; Dunlap, Urbanczyk, 2013; Thompson et al., 2004; Urbanczyk et al., 2011]. The list of luminescent bacteria is being expanded by both discovering new species [Ast et al., 2007; Cano-Gómez et al., 2010; Gomez-Gil et al., 2003; Lucena et al., 2012; Wang et al., 2010; Yoshizawa et al., 2009a, b, 2010a, b, 2012] and reclassification of long-known ones [Labella et al., 2017; Thompson et al., 2003; Urbanczyk et al., 2007]. However, due to anthropogenic load, the composition of biological species in ecosystems often changes. In many cases, the only ways to preserve natural biodiversity, *inter alia* that of microorganisms, are to carry out a detailed analysis of natural communities of luminescent bacteria by species and environmental criteria and to provide preservation of biological genotypes in collections.

Bioluminescent bacteria of the Black Sea and Sea of Azov remain poorly studied [Katsev, 2002; Katsev, Makemson, 2006; Maligina, Katsev, 2003; Tsybulskii, Sazykina, 2010]. Species composition of luminescent bacteria and their spatial distribution are of great interest due to features of these water areas. One of the key peculiarities of the Black Sea and Sea of Azov is the fact that many rivers flow into them; this results in lower salinity compared to values for other seas and oceans (16–18‰ for the Black Sea and 10–13‰ for the Sea of Azov).

A distinguishing feature of these water areas is noticeable seasonal variation in temperature which is more typical for the shallow Sea of Azov. This sea is also characterized by higher values of water temperature in summer, which, along with low salinity, leads to higher biological activity and more significant biodiversity compared to those for the Black Sea. Therefore, the aim of this work was to study species composition, biochemical properties, and characteristics of the distribution of luminous bacteria in coastal waters of the northern Black Sea and Sea of Azov.

MATERIAL AND METHODS

Sampling. To isolate bacteria in the northern coastal zone of the Black Sea and Sea of Azov, seawater and various hydrobionts were sampled May to October 2016–2018 (Fig. 1). The samples were placed in sterile containers and transported to a laboratory for further processing, which was carried out no later than 24 h after sampling. Details of the sampling location and their characteristics are provided in Table 1.

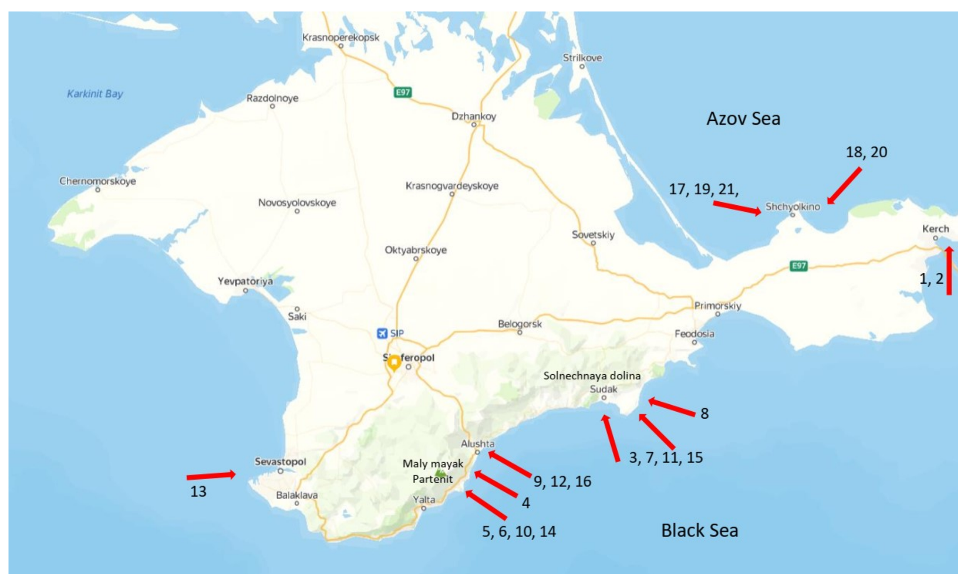

Fig. 1. Sampling sites for the isolation of luminescent bacteria

Рис. 1. Места отбора образцов для выделения люминесцентных бактерий

Table 1. Description of sampling sites

Таблица 1. Места отбора проб и их характеристики

Site No.	Sample (water/hydrobiont)	Site	Location	
The Black Sea				
1	Water	Kerch city	N45.254692°	E36.430439°
2	Water	Kerch city	N45.230556°	E36.414444°
3	Water	Sudak city	N44.840932°	E34.964564°
4	Water	Malyi Mayak village	N44.603173°	E34.372549°
5	Water	Partenit village	N44.559875°	E34.346913°
6	Water	Partenit village	N44.572081°	E34.346930°
7	Water	Sudak city	N44.831895°	E34.987922°
8	Water	Solnechnaya Dolina village	N44.863852°	E35.138874°
9	Water	Alushta city	N44.647252°	E34.401669°
10	Water	Partenit village	N44.561823°	E34.347663°
11	The Mediterranean mussel *Mytilus galloprovincialis*	Sudak city	N44.816742°	E35.049346°
12		Alushta city	N44.633788°	E34.392279°
13		Sevastopol city	N44.441391°	E33.640501°
14		Partenit village	N44.549120°	E34.347217°
15	The horse mackerel *Trachurus trachurus*	Sudak city	N44.797715°	E35.070433°
16	The brown shrimp *Crangon crangon*	Alushta city	N44.696810°	E34.444220°
The Sea of Azov				
17	Water	Shchelkino town	N45.426850°	E35.809528°
18	Water	Shchelkino town	N45.445819°	E35.846986°
19	Gobiidae gen. sp. (Pisces)	Shchelkino town	N45.451294°	E35.820341°
20	The Mediterranean green crab *Carcinus aestuarii*	Shchelkino town	N45.452265°	E35.852640°
21	Amphipoda fam. gen. sp.	Shchelkino town	N45.416109°	E35.791470°

Isolation of luminescent bacteria. After transportation to the laboratory, water samples were concentrated by filtration through a 0.45-μm membrane filter (Sartorius AG, Germany). The volume of the filtration sample varied within 10–50 mL depending on seawater temperature. After sample concentration, the filter was placed on the surface of solid media (HiMedia, India) containing 3% of sodium chloride. In summer (July and August), plating of water on a solid medium was carried out without *prior* concentration. In total, 200–500 μL of samples were applied to the surface of the nutrient agar in a Petri dish. The samples were incubated at +15…+25 °C, with periodical visual analysis of the results in a dark room. Upon detection of luminescent spots on the surface of the nutrient medium, pure bacterial culture was isolated by standard microbiological techniques.

Also, experimentally designed selective media based on water salinity in the sampling site were used for isolation of luminescent bacteria [Patent 2358009 RU, 2009; Patent 2368658 RU, 2009]. Bioluminescent bacteria were isolated, and the media were prepared as described in [Tsybulskii, Sazykina, 2010]. To isolate luminescent bacteria from fish samples, pieces of biomaterial less than 1–2 cm in size were placed in a sterile container and ⅔ covered with 3% solution of sodium chloride. Following incubation, bioluminescence analysis and bacteria isolation were carried out as described above. When using other hydrobionts as sources for bacteria isolation, those were removed from their shells (mussels), or chitinous exoskeleton (crabs and shrimps) was broken; then, biological material (samples 1–2 cm

in size) was processed as described above using 3% solution of sodium chloride. The isolated pure cultures of luminescent bacteria were stored as museum cultures in semi-liquid agar under a vaseline oil layer.

Identification of luminescent bacteria. Bacteria were identified by standard microbiological techniques. Morphological properties of bacteria, as well as their growth and bioluminescence characteristics, were evaluated at different temperatures (+10, +20, +25, +30, +35, +37, and +44 °C) and NaCl contents (0.5, 1, 2, 3, 4, 5, 6, 7, and 8%). Also, their enzymatic properties and ability to ferment various sugars (maltose, D-mannitol, and sucrose) were examined.

Bioluminescence kinetics. To study the kinetics of the luciferase reaction, enzyme preparations isolated from biomass of luminescent bacteria were used. Bacteria were cultivated, and the biomass was accumulated on a liquid nutrient medium under constant stirring at the optimal for each isolate temperature for 24 h. Bacterial cells were separated from the medium by centrifugation at 5,000 rpm for 30 min. The obtained biomass was washed with a 3% sodium chloride solution; then, it was suspended in a 0.01 M phosphate buffer in the ratio of 1 / 10 (biomass / buffer solution), pH = 7.0, at +4 °C. Cell destruction was carried out by 3-fold freezing–thawing, additionally using ultrasonic treatment and avoiding temperature increase of the samples to values above +15 °C. Leftover cellular debris was separated by centrifugation at 5,000 rpm for 30 min. The enzyme preparation containing luciferase was isolated from the resulting supernatant by ammonium sulphate precipitation, 25–80% of saturation.

To evaluate the kinetic characteristics of luciferase reaction, the protein precipitate obtained at the previous stage was dissolved in 0.1 M phosphate buffer, pH of 7.0. Then, 500 μL of 0.1 M phosphate buffer, pH of 7.0, 20–50 μL of the enzyme preparation (working dilutions were selected experimentally for each strain separately), and 20 μL of the 0.001% aqueous suspension of dodecanal (Sigma-Aldrich) were mixed in a chemiluminometer cuvette. The suspension of aldehyde was prepared by solvent exchange method. Dodecanal solution in ethanol was mixed with water in ratio 1 : 100. Luciferase reaction was initiated by adding 400 μL of photoreduced $FMNH_2$ (Sigma-Aldrich) at the concentration of 5×10^{-5} M containing 1×10^{-3} M of Trilon B. Bioluminescent signal was registered for 5 min after adding $FMNH_2$ till complete luminescence decay. The obtained graphical dependence of bioluminescence intensity on time was used for calculating the constant of the first-order bioluminescence decay rate (k, s^{-1}):

$$k = (\ln I_0/I)/t ,$$

where I_0 and I are intensity of bioluminescence at the initial moment and after the time period t, respectively.

Also, according to the diagrams, half-decay time of bioluminescence ($t_{½}$, s) was determined. Bioluminescence intensity dependence on time was recorded with a chemiluminometer Lum 100 (DISoft Ltd, Russia).

Molecular characteristics. Isolation of genomic DNA from microbial isolates. For the purpose of molecular genetic identification, total genomic DNA was isolated from the isolates. To isolate genomic DNA, the overnight culture of microorganisms was grown in 50-mL Erlenmeyer flasks on a liquid LB medium with addition of 3% NaCl. Twenty mL of medium was introduced into a flask and cultivated for 18 h in an incubator shaker at +25 °C and 170 rpm. Bacterial cells were precipitated by centrifugation in 2-mL screw-cap microtubes at 6,000 g for 2 min; ~ 75 mg of glass beads, 0.25 mm in diameter, was added to the obtained precipitate.

Then, 350 μL of guanidine solution (guanidine HCl 240 mM), 350 μL of detergent solution (Tris-HCl 500 mM, pH 8.0; SDS 2%, laurylsarcosinate 4%), and 400 μL of phenol-chloroform mixture were introduced into the tubes. Cells were destroyed by shaking on a laboratory vibrating mill Mixer Mill MM 400 (Retsch, Germany) for 2 min with a shaking frequency of 30 Hz. After that, the tubes were centrifuged for 7 min at 14,000 g; supernatant was taken; and 400 μL of chloroform

was added to it and thoroughly mixed with a vortex. Then, it was centrifuged as in the previous step. Subsequently, the aqueous phase was separated, and DNA was precipitated with an equal volume of isopropanol. The precipitate was washed twice with 70% ethanol, dried, and dissolved in 100 μL of deionized water. Discrete PCR amplicon bands were resolved on agarose gel. The PCR amplicon was purified to remove contaminants by the Cleanup Standard kit (Evrogen, Russia).

16S rRNA, *recA*, and *gyrB* gene amplification. To identify the isolated strains, amplification of 16S rRNA gene, as well as housekeeping genes encoding recombinase A (*recA*) and DNA gyrase B subunit (*gyrB*), was carried out. It was followed by determination of their nucleotide sequence. Sequencing of 16S rRNA gene and *gyrB* and *recA* housekeeping genes was performed by Sanger method [Sanger et al., 1977]. The structure of the primers used to obtain the target amplicons was taken from [Ast et al., 2009] and is shown in Table 2. Comparison of the sequence data and their differentiation was carried out using BLAST (https://blast.ncbi.nlm.nih.gov/). Phylogenetic relationships among submitted species were examined by means of MEGA X [Kumar et al., 2018]. Phylogenetic tree was constructed by neighbor-joining. A bootstrap analysis to investigate the stability of the tree was performed in 1,000 replicates. Also, the sequences of the following reference strains (https://www.ncbi.nlm.nih.gov) were added to the phylogenetic tree for comparison: *Vibrio harveyi* SB1 (NZ_CP125875.1), *Vibrio campbellii* BoB-53 (NZ_CP026321.1), *Photobacterium leiognathi* subsp. *mandapamensis* Lk8.2 (NZ_CP131594.1), *Aliivibrio wodanis* Vw11 (LR813705.1), and *Aliivibrio logei* 6Go0121 (MZ005969.1).

Table 2. The structure of the primers used to obtain the target amplicons

Таблица 2. Структура праймеров, используемых для получения целевых ампликонов

Gene	Forward primer sequence (5’-3’)	Reverse primer sequence (5’-3’)	Amplicon size (bp)
16S rRNA	AGAGTTTGATCCTGGCTCAG	TACGGYTACCTTGTTACGACTT	~ 1,500
recA	TCAAATTGAAAAACAATTTGGTAAAGG	ATCTTATCACCATTGTAGCTGTACC	~ 900
gyrB	GAAGTTATCATGACGGTACTTC	AGCGTACGAATGTGAGAACC	~ 1,200

To amplify the target genes, the following PCR modes were used. For 16S RNA gene, +95 °C – 2 min, 35 cycles (+95 °C – 20 s; +48 °C – 15 s; and +72 °C – 1 min); +72 °C – 5 min. For *recA* gene, +95 °C – 2 min, 35 cycles (+94 °C – 20 s; +45 °C – 15 s; and +68 °C – 1 min); +72 °C – 7 min. For *gyrB* gene, +95 °C – 2 min, 35 cycles (+95 °C – 20 s; +49 °C – 15 s; and +72 °C – 1 min); +72 °C – 5 min. Amplicons were purified by cutting an agarose gel (1% agarose, TBE buffer, 6 $V \cdot cm^{-1}$) strip stained with SYBR Green (DNK-Sintez, Russia). The target product was isolated from the agarose gel using the Cleanup Standard kit (Evrogen, Russia) according to the manufacturer’s instructions. The amplified fragments of 16S rRNA, *recA*, and *gyrB* genes were sequenced at Evrogen company (Moscow).

RESULTS AND DISCUSSION

Field studies conducted May to October 2016–2018 in various coastal areas of the Black Sea and Sea of Azov (Fig. 1) allowed us to identify 21 bacterial isolates with visible bioluminescence. In the spring–summer season (May to June), when the Black Sea water had not yet fully warmed up, and its temperature averaged +16…+20 °C, the abundance of luminescent bacteria was low. Accordingly, the bacteria were isolated with preliminary concentration on filters. In summer (July to August), seawater temperature in the Black Sea reached +25 °C, and luminescent forms of bacteria were plated from water without additional concentration of samples. Isolation and research on luminescent bacteria of the Sea of Azov were carried out in August, when seawater temperature reached +30 °C. Luminescent forms occurred everywhere and were isolated both from seawater and various marine hydrobionts.

Table 3 provides biochemical characteristics of cultured luminescent bacteria strains isolated from water and hydrobionts of the Black Sea and Sea of Azov. For isolated strains, sequence data on 16S rRNA gene and *gyrB* and *recA* housekeeping genes were obtained (GenBank accession numbers were MK692515–MK692535). The results of phylogenetic analysis based on the comparison of 16S rRNA genes sequences are presented in Fig. 2 (compared to some reference bacteria strains). The data on molecular genetic identification are also provided in Table 3. The analysis revealed the presence of three well-supported clades. All the isolated luminous strains tested here resolved unambiguously either to the *Vibrio* clade, or to the *Aliivibrio* clade, or to the *Photobacterium* clade. Cells of all isolated strains are gram-negative. They are capable of growing at +15…+35 °C, with the optimum of +20…+30 °C, at 0.5–5.0% NaCl (weight/volume, w/v), with the optimum of 1.5–3.0 %, and at pH 6.0–8.0, with the optimum of 7.0–8.0. All of them are able to ferment glucose and mannose with acids formation.

Table 3. Results of identification of cultivated strains of bioluminescent bacteria isolated from the Black Sea and Sea of Azov

Таблица 3. Результаты идентификации культивируемых штаммов биолюминесцентных бактерий, выделенных из Чёрного и Азовского морей

No.	Result of molecular genetic identification	Kinetics of luciferase reaction			Fermentation of sugars		
		k, s^{-1}	$t_{½}$, s	type	maltose	D-mannitol	sucrose
The Black Sea							
The genus *Vibrio*							
1	*Vibrio campbellii*	0.038	18.2	S	+	+	–
2	*Vibrio harveyi*	0.040	17.3	S	+	+	+
3	*Vibrio jasicida*	0.036	19.3	S	+	+	–
4	*Vibrio jasicida*	0.037	18.7	S	+	+	–
5	*Vibrio aquamarinus* sp. nov.	0.038	18.2	S	+	+	–
6	*Vibrio campbellii*	0.036	19.3	S	+	+	–
7	*Vibrio jasicida*	0.043	16.1	S	+	+	–
8	*Vibrio campbellii*	0.050	13.9	S	+	+	–
9	*Vibrio aquamarinus* sp. nov.	0.045	15.4	S	+	+	–
10	*Vibrio owensii*	0.059	11.7	S	+	+	+
11	*Vibrio aquamarinus* sp. nov.	0.040	17.3	S	+	+	+
12	*Vibrio owensii*	0.041	16.9	S	+	+	+
13	*Vibrio campbellii*	0.059	11.7	S	+	+	+
14	*Vibrio owensii*	0.056	12.4	S	+	+	+
The genus *Aliivibrio*							
15	*Aliivibrio fischeri*	0.38	1.8	F	+	–	–
16	*Aliivibrio fischeri*	0.43	1.6	F	+	–	–
The Sea of Azov							
The genus *Photobacterium*							
17	*Photobacterium leiognathi*	0.45	1.5	F	–	–	–
18	*Photobacterium leiognathi*	0.48	1.4	F	–	–	–
19	*Photobacterium leiognathi*	0.37	1.9	F	–	–	–
20	*Photobacterium leiognathi*	0.42	1.7	F	–	–	–
21	*Photobacterium leiognathi*	0.36	1.9	F	–	–	–

Note: S, slow-type luciferase kinetics; F, fast-type luciferase kinetics.

Примечание: S — кинетика люциферазы медленного типа; F — кинетика люциферазы быстрого типа.

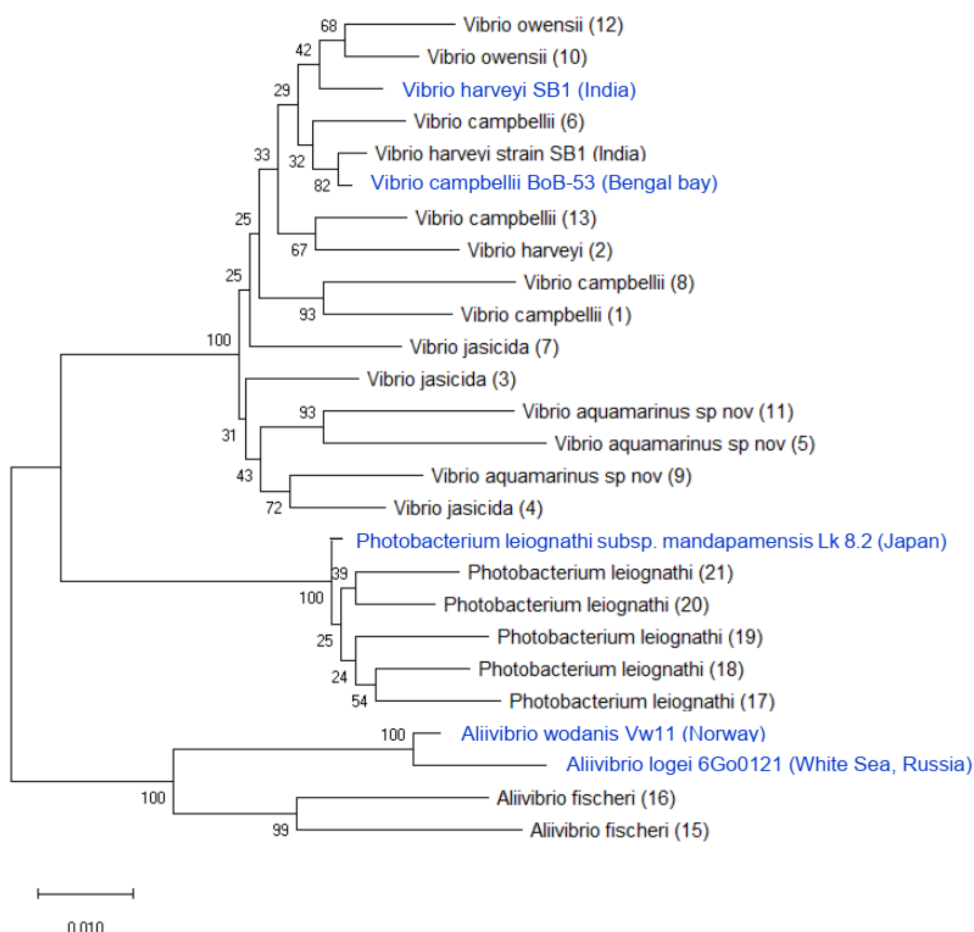

Fig. 2. Phylogenetic tree based on the analysis of 16S rRNA sequences, constructed by neighbor-joining [Saitou, Nei, 1987]. Bootstrap percentages from 1,000 replicates appear next to respective branches. The scale bar indicates the number of inferred nucleotide changes. The strain numbers (1–21) correspond to the numbers given in Table 3

Рис. 2. Филогенетическое дерево, основанное на анализе сиквенсов 16S rRNA, которое построено по методу присоединения соседей (neighbor-joining) [Saitou, Nei, 1987]. В узлах ветвей указан индекс (%) бутстреп-анализа для 1000 повторов. Масштабная линейка показывает количество предполагаемых замен нуклеотидов. Номера штаммов (1–21) соответствуют номерам в табл. 3

The following criteria and recommendations were used for primary identification of isolated bacteria. The presence of yellow pigment and the fast-type luciferase kinetics, as well as the ability of maltose fermentation, determined the bacteria species *A. fischeri* [Farmer III, Michael Janda, 2015; Farmer III et al., 2015]. These strains had average range of substrate specificity (3 out of 5 studied carbohydrates). Other isolates capable of fermenting maltose and D-mannitol, containing no yellow pigment, and characterized by the slow-type luciferase kinetics were assigned to the genus *Vibrio* [Farmer III, Michael Janda, 2015; Farmer III et al., 2015]. Bacteria not capable of fermenting D-mannitol and sucrose, having no yellow pigment, and characterized by the fast-type luciferase kinetics were classified as *Photobacterium*. Isolated strains of this genus capable of growing at +30 °C and non-fermenting maltose were assigned to *Photobacterium leiognathi* [Moi et al., 2017; Thyssen, Ollevier, 2015]. Later, molecular genetic identification of the studied isolates confirmed their belonging to indicated genera.

The obtained data revealed the following phenotypic features of the strains. All the Black Sea *Vibrio* strains isolated from mussels (isolates 11–14) had the slow-type luciferase kinetics and the ability to ferment D-mannitol and sucrose. On the other hand, vibrions obtained from seawater in various coastal zones of the Black Sea (isolates 1–10) were characterized by the slow-type luciferase kinetics

and the ability to ferment D-mannitol, but only 2 out of 10 strains utilized sucrose: *V. owensii* and *V. harveyi*. None of *V. jasicida* isolates fermented sucrose; among the isolated *V. campbellii* and *V. aquamarinus* strains, there were ones capable and incapable of utilizing sucrose. Thus, all *Vibrio* isolates had the slow-type luciferase kinetics and an extended range of utilized sugars (4–5 out of 5 studied). This coincides with the *Bergey's Manual* data: as indicated there, only 83% of strains of this genus have the ability to utilize sucrose [Baumann, et al., 1984; Farmer III, Michael Janda, 2015; Farmer III et al., 2015].

Cultural, biochemical, and genetic identification of luminescent bacteria isolated from water and hydrobionts of the Sea of Azov showed that all of them belong to *P. leiognathi*. This species has bright bioluminescence, the fast-type luciferase kinetics, and a narrow range of utilized substrates (2 out of 5 studied sugars). It was practically not encountered during the study of samples of the Black Sea water and hydrobionts. Apparently, high temperature of seawater, its low salinity, and, consequently, high biological activity of the sea cause the predominance of this species in the Sea of Azov water and also lead to the colonization of hydrobionts inhabiting it.

Fig. 3 provides the results of occurrence of different types of cultivated luminescent bacteria isolated in the studied water areas of the Black Sea and Sea of Azov. The ratio of isolated strains was as follows: *V. harveyi*, 4.76%; *A. fischeri*, 9.52%; *V. jasicida*, 14.29%; *V. aquamarinus* sp. nov., 14.29%; *V. owensii*, 14.29%; *V. campbellii*, 19.04%; and *P. leiognathi*, 23.81%.

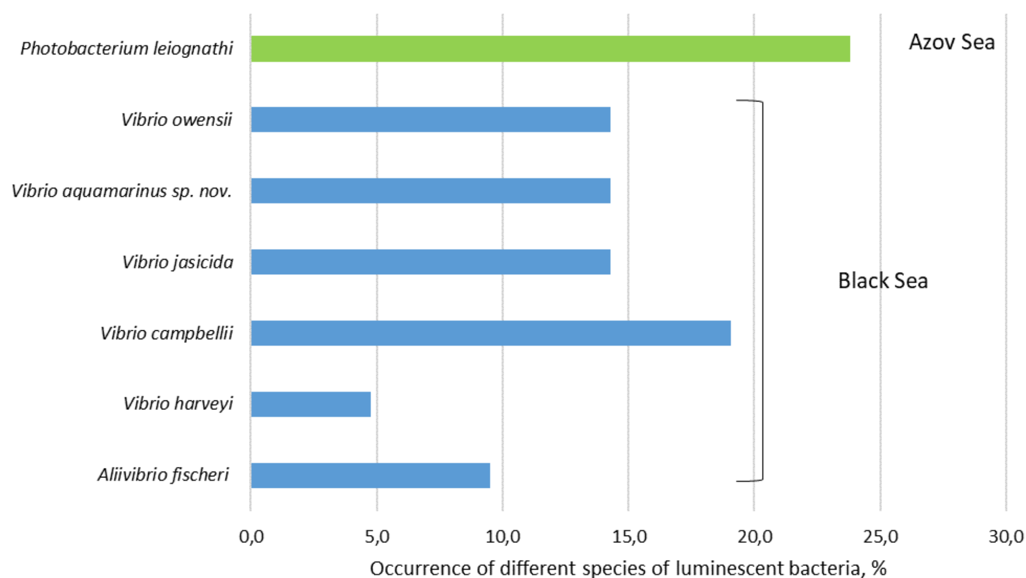

Fig. 3. Occurrence (%) of different species of cultivated luminescent bacteria isolated from water and hydrobionts of the Black Sea and Sea of Azov

Рис. 3. Встречаемость (%) различных видов культивируемых люминесцентных бактерий, выделенных из воды и гидробионтов Чёрного и Азовского морей

Concerning the object of isolation, it should be noted as follow: 9 isolates were isolated from hydrobionts, and 12, from seawater. Strains isolated from seawater belong to the species *V. harveyi* and *V. jasicida*. *Aliivibrio fischeri* strains were isolated from hydrobionts alone, while *V. campbellii*, *V. owensii*, and *V. aquamarinus* sp. nov. strains were isolated from both water and hydrobionts. Interestingly, all the strains of the genus *Vibrio* isolated from hydrobionts had the ability to utilize sucrose, while *V. campbellii* and *V. aquamarinus* sp. nov. isolated from seawater did not ferment sucrose. Apparently, in these species, this ability is associated with symbiosis and depends on the ecological niche occupied by a certain strain.

Notably, the new isolates confirmed the occurrence of a new species of the genus *Vibrio*, *V. aquamarinus*, in the Black Sea. Isolates are deposited in the Russian National Collection of Industrial Microorganisms (*V. aquamarinus* VKPM B-11245) and German Collection of Microorganism and Cell Culture (*V. aquamarinus* DSM 26054).

Conclusion. Studies have shown that a significant difference in environmental conditions between the Black Sea and Sea of Azov in summer leads to prevalence of different taxa of luminescent bacteria. The genus *Photobacterium* represented by the species *P. leiognathi* dominates the Sea of Azov characterized by low salinity and high water temperatures. The genus *Vibrio* represented by the species *V. campbellii*, *V. jasicida*, *V. harveyi*, *V. owensii*, and *V. aquamarinus* sp. nov. can be considered the prevailing genus of luminous bacteria that inhabit the Black Sea water and live in its mussels. The obtained results showed variability of *V. campbellii* and *V. aquamarinus* sp. nov. strains by the ability to ferment sucrose depending on the isolate source (water or hydrobionts). Investigations on cultivated luminescent bacteria of the northern Black Sea have also revealed the occurrence of *Aliivibrio fischeri* associated with various hydrobionts (pelagic fish and shrimps).

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БИОЛЮМИНЕСЦЕНТНЫЕ БАКТЕРИИ ЧЁРНОГО И АЗОВСКОГО МОРЕЙ

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Целью настоящего исследования было выделить биолюминесцентные бактерии из прибрежных акваторий Чёрного и Азовского морей, изучить их морфологические и биохимические характеристики и идентифицировать их на основе последовательностей генов 16S рРНК, *recA* и *gyrB*. Из морских гидробионтов выделены 9 изолятов, из морской воды — 12. Результаты биохимических и молекулярно-генетических исследований показали, что выделенные светящиеся бактерии относятся к родам *Vibrio*, *Aliivibrio* и *Photobacterium*. Установлено, что все 5 люминесцентных штаммов, выделенных из воды и гидробионтов Азовского моря, принадлежат виду *Photobacterium leiognathi*. Бактерии, выделенные из Чёрного моря, отнесены к родам *Aliivibrio* и *Vibrio*. Род *Aliivibrio* представлен 2 штаммами *Aliivibrio fischeri*, ассоциированными с различными гидробионтами; 14 штаммов рода *Vibrio* отнесены к видам *Vibrio campbellii*, *V. jasicida*, *V. harveyi*, *V. owensii* и *V. aquamarinus* sp. nov. Таким образом, таксономический состав культивируемых люминесцентных бактерий в Азовском и Чёрном морях существенно различается.

Ключевые слова: люминесцентные бактерии, идентификация, таксономический состав, биоразнообразие, Чёрное море, Азовское море